

a nucleic acid sequence that encodes the amino acid sequence of SEQ ID NO: 2, or
a nucleic acid sequence from *Alcaligenes*, which encodes D-aminoacylase and which
comprises the following sequence of restriction sites: EcoR I - Bgl II - Pvu II – Hind III;
wherein said microorganism is zinc resistant, and
wherein the expression of D-amino acylase from said nucleic acid sequence in said
microorganism is enhanced in the presence of zinc ion.

15. (New) The isolated microorganism of Claim 14 that comprises a nucleic acid
sequence that encodes SEQ ID NO: 2.

16. (New) The isolated microorganism of Claim 14 that comprises the nucleic acid
sequence of SEQ ID NO: 1.

17. (New) The isolated microorganism of Claim 14 that comprises a D-amino
acylase gene from *Alcaligenes*, the expression of the gene product of which is enhanced in the
presence of zinc ion, which encodes a D-aminoacylase and which comprises the following
sequence of restriction sites: EcoR I - Bgl II - Pvu II – Hind III.

18. (New) The isolated microorganism of Claim 14, wherein the D-aminoacylase-
producing gene is obtained from *Alcaligenes xylooxidans*, *subsp. xylooxidans* strain A-6.

19. (New) The isolated microorganism of Claim 14, wherein the D-aminoacylase-
producing gene is modified inserting a specific nucleotide sequence, GAAGGA, (SEQ ID
NO: 3) in the ribosome-binding site in the position of the ninth base upstream of the
translation initiation point of the gene.

20. (New) The isolated microorganism of Claim 14, wherein the D-aminoacylase-
producing gene is modified by:

creating a *Hind III* recognition site upstream and downstream from the D-
aminoacylase gene,

excising or purifying the resulting modified gene and
ligating the modified gene into an expression vector.

21. (New) The isolated microorganism of Claim 14, wherein the zinc tolerance of the host microorganism is such that the cell weight of the microorganism either increases, or decreases, within a range of 10% in a culture medium with 2 mM zinc added thereto on the basis of the cell weight measured at A660 nm in a zinc-free culture medium.

22. (New) The isolated microorganism of Claim 14, wherein the zinc tolerance of the microorganism is such that the cell weight of the microorganism either increases, or decreases, within a range of 20% in a culture medium with 5 mM zinc added thereto on the basis of the cell weight measured at A660 nm in a zinc-free culture medium.

23. (New) The isolated microorganism of Claim 14, which is *Escherichia coli*.

24. (New) A process for producing D-aminoacylase comprising:
culturing the isolated microorganism of Claim 14 in a culture medium containing zinc
and
recovering D-aminoacylase.

25. (New) The process of Claim 24, further comprising culturing said microorganism in a medium containing a *tac* promoter-inducing substance.

26. (New) The process of Claim 24, wherein said promoter-inducing substance is isopropyl thiogalactoside (IPTG) or lactose.

27. (New) The process of Claim 24, wherein said culture medium has a concentration of lactose ranging from 0.1 to 1%.

28. (New) An isolated nucleic acid sequence:

which encodes the amino acid sequence of SEQ ID NO: 2, or

which encodes a D-aminoacylase from *Alcaligenes*, and which comprises the

following sequence of restriction sites: EcoR I - Bgl II - Pvu II – Hind III.

29. (New) The isolated nucleic acid sequence of Claim 28, which encodes the amino acid sequence of SEQ ID NO: 2.

30. (New) The isolated nucleic acid sequence of Claim 28, which is a D-aminoacylase gene from *Alcaligenes*, which comprises the following sequence of restriction sites: EcoR I - Bgl II - Pvu II – Hind III.

31. (New) A vector comprising the nucleic acid sequence of Claim 28.

32. (New) An isolated nucleic acid sequence from *Alcaligenes* that encodes a D-aminoacylase and which comprises the following sequence of restriction sites: Sal I – Bgl II – Pvu II.

33. (New) A vector comprising the nucleic acid sequence of Claim 32.

34. (New) A zinc-resistant host cell comprising the nucleic acid sequence of Claim 32.

REMARKS

Claims 14-34 are active. Independent Claim 14 tracks and finds support in the original claims and in the specification. The amino acid sequence shown in SEQ ID NO: 2 is described in the original sequence listing (see original SEQ ID NO: 1). The restriction site sequence EcoR I – Bgl II- Pvu II – Hind III is described in original Fig. 2. Similarly, the Sal I – Bgl II – Pvu II restriction site sequence referred to in Claims 32-34 is described by Fig. 2. Claims 19-23, respectively, track Claims 6-10. Claim 24 finds support in original Claim 3. Claims 25-27, respectively track Claims 11-13. Claims 28-34 find support in original Fig. 2. Accordingly, the Applicants do not believe that any new matter has been added.